# PHENOTYPIC CHARACTERIZATION OF SOYBEAN BRADYRHIZOBIA IN TWO SOILS OF NORTH CAROLINA

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**Summary**—Serotypic composition of nodules (480 per soil type) from five soybean cultivars grown on two (Dothan and Cape Fear) soils of the Atlantic Coastal Plain of North Carolina was characterized. Symbiotic $N_2$-fixation efficiency, capacity for induction of foliar chlorosis symptomatic of rhizobitoxine production and antibiotic resistances of isolates purified from these nodules were also determined. While host plant cultivar had no significant effect on the serotype distribution, soil type had a large effect on the distribution and diversity of serotypes. Forty-six serotypes were identified among nodules from the Cape Fear soil, but only serotype 46/76 (8%), 76 (11%), 94 (9%) and 122/124 (12%) occurred in more than 5% of the nodules. Thirty percent of nodule occupants were not identified with the eleven antisera used. Twenty-four serotypes were identified among nodules from the Dothan soil. Of these serotypes 31/94 (32%), 46/76 (16%), and 76 (23%) occurred in more than 15% of the nodules. Five percent of the nodule occupants were not identified. Major serotypes did not change, but their frequency changed when fields were sampled at different growth stages in the same season and at the same growth stage in different seasons. Isolates serotyped as 31/94, 46/76, and most of the isolates serotyped as 76 generally exhibited higher levels of resistance to streptomycin and erythromycin than isolates serotyped as 24, 94 and 122/124. Five percent of the isolates from the Cape Fear soil (all serotyped as 31/94) and 18% of the isolates from the Dothan soil (serotyped as 31/94 or 76) induced foliar chlorosis when cultivar Brim was the host. Only 12–14% of the isolates from the two populations had $N_2$-fixation capacity equal to or greater than that of the efficient reference strain MN110. However, four improved soybean cultivars grown in the same fields and year that isolates were obtained did not exhibit a significant seed yield response to application of 150 kg N ha$^{-1}$ when yields in the minus N treatment ranged from 3.2 to 3.7 Mg ha$^{-1}$. A significant seed yield response by a non-nodulated cultivar indicated that these soils were N limited. Therefore, the symbiotic $N_2$-fixation capacity of these bradyrhizobial populations did not limit soybean seed yields despite the low percentage of isolates with high $N_2$-fixation efficiency and the presence of isolates with the capacity to induce leaf chlorosis symptomatic of rhizobitoxine production. Published by Elsevier Science Ltd

## INTRODUCTION

Inoculation with efficient bradyrhizobial strains has generally failed to increase $N_2$ fixation by soybean plants growing in N-limited soils with established bradyrhizobial populations. Typically the recovery of the inoculant strains in the nodules of such plants is low, and most of the nodules are formed by indigenous strains of bradyrhizobia (Ham *et al.*, 1971; Howle *et al.*, 1987). Although this phenomenon has been apparent for many years, there is limited research on the genetic and physiological properties of indigenous populations of soybean bradyrhizobia. Information about the $N_2$-fixation capacity, persistence and competitive ability of indigenous bradyrhizobial strains would help to evaluate the need for inoculation and the prospect of

selecting useful inoculant strains from indigenous populations.

While antibiotic resistance patterns (Mueller *et al.*, 1988; Sawada *et al.*, 1990), electrophoretic mobility of cellular proteins (Kamicker and Brill, 1986) and rhizobitoxine production (Fuhrmann, 1990) have been used to characterize bradyrhizobial strains in indigenous populations, serological reactions are the most common methods (Means *et al.*, 1964; Fuhrmann, 1990). Since bradyrhizobial strains with similar antigenic properties can be quite diverse with respect to other traits (van Berkum *et al.*, 1993), measurements of symbiotic $N_2$-fixation efficiency, antibiotic resistances and capacity for induction of leaf chlorosis should be used to characterize the diversity among isolates from soil populations. Evaluation of these additional traits also would allow classification of strains that do not react with any of the available antisera.

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Mpepereki and Wollum (1991) evaluated the serotypic diversity of soybean bradyrhizobial populations in nodules sampled once during the season within and amongst six fields in the Coastal Plain and Piedmont of North Carolina. Serotype and its proportions varied among locations. Relative to the reference strain USDA 110, 42% of the isolates from these nodules had significantly lower symbiotic $N_2$-fixation efficiencies, while only 1% had significantly greater efficiencies (S. Mpepereki, *pers. commun.*). While yields of soybean crops from these fields were not evaluated, Singleton and Tavares (1986) reported that neither symbiotic $N_2$ fixation nor yield was enhanced when competition by native strains was overcome with selected inoculant strains when the soil populations were above a threshold number and had some effective strains. An assessment of the capacities of soil populations of bradyrhizobia to fix sufficient $N_2$ to optimize seed yield would thus improve the basis for interpreting genetic and physiological data obtained for isolates derived from these populations.

The objective of this study was to relate physiological properties of isolates from bradyrhizobial populations of two soils to the capacity of the total soil populations to fix sufficient $N_2$ to remove N availability as a seed yield constraint under the existing environmental conditions. To achieve this objective, nodule occupants from non-inoculated soybeans were evaluated for serotype, symbiotic $N_2$-fixation capacity, antibiotic resistances and capacity to induce foliar chlorosis. The seed yields of plants dependent on residual soil N and on symbiotic $N_2$ fixation by bradyrhizobial strains from the indigenous populations were compared to seed yields of plants supplied with 150 kg N ha$^{-1}$ as ammonium nitrate to determine whether the collective symbiotic $N_2$-fixation efficiency of nodule occupants from the indigenous populations was a yield-limiting factor.

### MATERIALS AND METHODS

*Experimental design*

The soybean (*Glycine max* L. Merr.) cultivar Arksoy and its descendants Davis, Young, Brim, N90-852, and non-nodulating Lee were grown in 1991 on a Dothan (Plinthic Kandiudult, fine loamy, siliceous, thermic) soil at the Central Crops Research Station near Clayton, NC and on a Cape Fear (Typic Umbraquult, clayey, mixed, thermic) soil at the Tidewater Research Station near Plymouth, NC. The most notable difference between these soils is the organic matter content of 4.8% for the Cape Fear and 0.4% for the Dothan soil (Table 1). The Cape Fear soil had been cropped with soybean every second or fourth year in rotation with corn since the land had been cleared from secondary forest in the late 1970 s and the

Table 1. Summary of selected soil properties

| Property* | Soil | |
|---|---|---|
| | Cape Fear | Dothan |
| Humic matter (%) | 4.8 | 0.4 |
| Bulk density (g cm$^{-3}$) | 0.96 | 1.44 |
| CEC (meq dm$^{-3}$) | 8.3 | 2.0 |
| Base saturation (%) | 71 | 100 |
| Acidity (meq dm$^{-3}$) | 2.4 | 0.0 |
| pH | 5.5 | 6.3 |

*All values were obtained from reports of soil tests conducted by the North Carolina Department of Agriculture, Soil Test Division, Raleigh NC.

Dothan soil had been cropped with a 3 y rotation of corn–tobacco–soybean for at least 12 y. No inoculant was used at planting; hence, in minus-N plots, plants were dependent on soil N reserves and $N_2$ fixation by bradyrhizobia indigenous to the soils. In addition to minus-N treatments, each cultivar was grown in plots to which a total of 150 kg N ha$^{-1}$ as ammonium nitrate was applied in three equal doses at late vegetative, beginning podfill and mid podfill growth stages. Plots were composed of six 5.8 m long rows. Half of each minus-N plot was used for measurement of seed yield and the other half for sampling of nodules for serological analysis. The experiments were arranged in a randomized complete block design with four replications of each cultivar by N treatment combination.

In 1993, the serological properties of indigenous bradyrhizobia infecting the cultivar Young were determined at two sampling dates (vegetative and beginning podfill) in the same soils used in 1991. The experiment with the Cape Fear soil was conducted in the same field as in 1991. The Dothan soil was in a field 270 m from and in the same drainage basin as the one used in 1991. Plots of non-inoculated plants were arranged in a randomized complete block design with four replications. Serological data collected from this experiment and from the 1991 experiment allowed an assessment of the effect of season and plant growth stage on the distribution of serotypes in nodules.

*Nodule collection and analysis*

In 1991, six plants were collected from each minus-N plot during vegetative growth (40–50 d after planting, DAP). Nodules were excised and mixed to form a composite sample. A subsample of 24 nodules from each replicate was disinfected with 95% v/v ethanol for 1 min, 1% v/v Na hypochlorite for 3–4 min and rinsed five times with sterile water. Disinfected nodules were crushed in 2 ml sterile NaCl (0.85%, w/v). Part of the nodule macerate was streaked on yeast extract-mannitol (YEM) plates (Vincent, 1970) and the remainder of the macerate was used for serological analysis. Single colonies were picked and streaked on YEM agar plates and purified by resuspending cells in a 0.01%

v/v Tween 20 solution and restreaking on YEM plates. Isolates were maintained on YEM slants and in water suspensions (Crist et al., 1984), for further characterization.

A subsample of 43 isolates from the Cape Fear soil and 44 from the Dothan soil were further characterized for antibiotic resistance, $N_2$-fixation capacity and induction of foliar chlorosis. The number of isolates representing each serotype was approximately proportional to the mean frequency observed in nodules sampled in 1991 and 1993 (vegetative growth stage).

In 1993, a composite nodule sample was obtained during vegetative growth (56 DAP for the Cape Fear and 46 DAP for the Dothan) and at early podfill (102 DAP for the Cape Fear soil and 92 DAP for the Dothan soil). At each sampling 16 nodules from each replicate were used for the serological analysis.

### Serological analysis (ELISA)

Samples were analyzed by an indirect enzyme-linked immunosorbent assay (ELISA), as described by Fuhrmann and Wollum (1985). Nodule macerates were diluted with water to an absorbance ($A_{600}$) of 0.5 units. Flagellar antigens were inactivated by heating the suspensions at 100°C for 10 min. Each sample was analyzed with antisera produced against the following strains of B. japonicum: USDA 24, USDA 31, USDA 46, USDA 76, USDA 94, USDA 110, USDA 122, USDA 124, NC 1001, NC 1028 and NC 1033. Absorbance readings were recorded when the absorbance ($A_{410}$) of a known reference antigen in a plate reached ca. 1.8 absorbance units. Unknown antigens were considered to have a positive reaction if their absorbances were: (1) 40% of that of a nodular suspension of the strain homologous to the respective antiserum, and (2) more than 0.5 absorbance units above the reading of the blank (wells that were not coated with antigens). Statistical comparisons of the serotype distributions in the two soils were made using the Chi-square test (Steel and Torrie, 1985). The percentages of five serotypes that were common to both soils (31/94, 46/76, 76, 94, and 122), the percentages of nodules from both soils that did not react with any of the antisera (NI) and the percentages of minor serotypes pooled into one group for each soil were used in the analysis.

### Antibiotic resistance

Antibiotic resistance was determined as described by Somasegaran and Hoben (1985). Isolates were inoculated into YEM broth and grown to $10^9$ colony-forming units (CFU) per ml$^{-1}$. Dilutions containing $10^6$ CFU ml$^{-1}$ were streaked on duplicate YEM plates containing streptomycin or spectinomycin at concentrations of 13, 25, 50, 100, 200, 400 or 800 $\mu$g ml$^{-1}$, or erythromycin in concentrations

of 13, 25, 50, 100, 200 or 400 $\mu$g ml$^{-1}$. A YEM plate without antibiotic was inoculated as a control. After incubation for a week at 28°C, the growth was rated as positive or negative. The minimal inhibitory concentration (MIC) was expressed as the lowest concentration of an antibiotic ($\mu$g ml$^{-1}$) for which no bacterial growth was observed.

### $N_2$-fixing capacity and induction of leaf chlorosis

Plastic cups (1 l) containing vermiculite were flushed twice with water and once with N-free nutrient solution (McClure and Israel, 1979), just before sowing the seeds. Since tests had revealed that cultivar Brim is subject to leaf chlorosis symptomatic of rhizobitoxine production by nodule symbionts, it was used as the host plant for characterization of isolates. Seeds were disinfected with ethanol (95%, v/v) for 1 min and sodium hypochlorite (1%, v/v) for 3 min, followed by six rinses with sterile water. Three seeds, each inoculated with 1 ml of a broth culture containing ca. $10^9$ CFU ml$^{-1}$, were sown in each cup. A non-inoculated control was also included. From 3 to 7 DAP, either 20 ml of water or 20 ml of N-free nutrient solution were added on alternate days. The plants were thinned to one per cup at 7 DAP and organized in a randomized complete block design with four replications. During the rest of the experiment the pots received 100 ml of water daily. From 7 to 28 DAP, 100 ml of N-free nutrient solution was supplied on alternate days following the application of water and beyond 28 DAP, 100 ml of N-free nutrient solution was applied daily after the application of water.

At 36 DAP, the shoot appearance was evaluated for general chlorosis symptoms (plants light green all over) or for presumptive rhizobitoxine-induced chlorosis (i.e. chlorosis limited mostly to the apical meristem and recently-formed leaves with a light yellow to almost white color, Owens and Wright, 1965). Shoots were clipped just below the cotyledonary node, dried at 60°C for 3 d, and weighed.

Shoot N content was analyzed by a modified Kjeldahl method employing a zirconium–copper catalyst (Glowa, 1974) and a salicylic acid pre-digestion step (Nelson and Sommers, 1973). The total amount of $N_2$ fixed was estimated by subtracting total N in shoot of non-inoculated control plants from total N in shoot of inoculated plants. Nitrogen-fixing efficiency was estimated by comparing total $N_2$ fixed by each isolate with that fixed by two control strains: MN110 (Mathis et al., 1986) and USDA 31. Isolates that fixed equal or greater amounts of $N_2$ than strain MN110 were classified as having high $N_2$-fixation capacity, while those that fixed equal or lower amounts of $N_2$ than strain USDA 31 were classified as having low $N_2$-fixation capacity. Isolates that fixed lower amounts of $N_2$ than strain MN110 but more than strain USDA 31

were classified as having intermediate $N_2$-fixation capacity. The effect of bradyrhizobial symbiont on $N_2$ fixed was evaluated using the ANOVA procedure of the Statistical Analysis System (SAS Institute Inc., 1982). Protected LSD values ($P = 0.05$) were calculated and used as the criterion for the symbiotic-capacity classification.

### Yield measurements

Seeds were harvested from 4.9 m of bordered row with a plot combine and weighed. Seed yields were adjusted to 13% moisture. The effects of cultivar, N supply and the interaction between these factors on seed yield were tested using the ANOVA procedure of the Statistical Analysis System (SAS Institute Inc., 1982). Since effects of cultivar, N supply and the interactions between these two factors were significant at the 0.05 probability level, $LSD_{05}$ values were calculated for comparison of any two treatment means.

### RESULTS

### Antigenic properties

Nodules were classified into specific serotypes based on reactions with a set of antisera prepared against 11 reference strains (for example serotype 31/76 refers to nodule macerates that react with antisera raised against reference strains USDA 31 and USDA 76). Chi-square analysis indicated no significant difference ($P = 0.05$) in the distribution of serotypes in nodules among the five soybean cultivars grown at either site. Thus, data were pooled by sites for subsequent analysis (Table 2).

Chi-square analysis of serotype distributions based on analysis of 480 nodules per soil revealed significant differences ($P = 0.05$) in the serological composition of the two soil populations of bradyrhizobia (Table 2). For the Cape Fear soil, each cultivar was infected by 16–21 serotypes. A total of 46 different serotypes was observed, of which 25 occurred in only one or two nodules from the total sample (480 nodules, data not shown). Serotypes 46/76, 76, 94, and 122/124 occurred in more than 3% of the nodules tested (Table 2). Other serotypes, which individually occupied less than 3% of the nodules, collectively accounted for 26% of the nodules tested. A large percentage of the nodule suspensions (30%) did not react with any of the antisera used.

For the Dothan soil, 11–14 serotypes were found in each cultivar and a total of 24 serotypes was observed, of which 13 occurred in only one or two nodules (data not shown). A high percentage of the nodules was occupied by the four serotypes 31/94, 46/76, 76, and 94. Other identified serotypes occupied 17% of the nodules, with each representing less than 5% of the total nodules. Only 5% of the

Table 2. Distribution of major serotypes in nodules of soybean plants grown in a Cape Fear and a Dothan soil in 1991*

| Serotype | Percentage in nodules[†] | |
|---|---|---|
| | Cape Fear | Dothan |
| NI[‡] | 30 | 5 |
| 24 | 2 | 3 |
| 31/76 | 0 | 4 |
| 31/94 | 4 | 32 |
| 46/76 | 8 | 16 |
| 76 | 11 | 23 |
| 94 | 9 | 7 |
| 122/124 | 12 | 2 |
| 122/124/1033 | 3 | 0 |
| 124 | 2 | 0 |
| 1001 | 3 | 0 |
| 1001/1028 | 2 | 0 |
| Minor serotypes[§] | 14 | 8 |

*Serotypes NI, 31/94, 46/76, 94, and 122/124 were considered as separate categories in the $\chi^2$ analysis. All other serotypes were pooled by site to form one additional category for the analysis. The distribution of serotypes was significantly different between the two soils ($P = 0.05$).
[†]Mean for five cultivars and a total of 480 nodules per soil.
[‡]NI: not identified.
[§]Minor serotypes: individual serotypes present in less than 2% of the nodules analyzed.

nodule suspensions did not react with any of the antisera used.

In the first 1993 nodule sampling in the Cape Fear soil, the major serotypes were the same as in 1991: 31/94, 46/76, 76, 94 and 122/124 (Fig. 1A). However, $\chi^2$ analysis comparing percentages of these serotypes, of non-identified serotypes and of
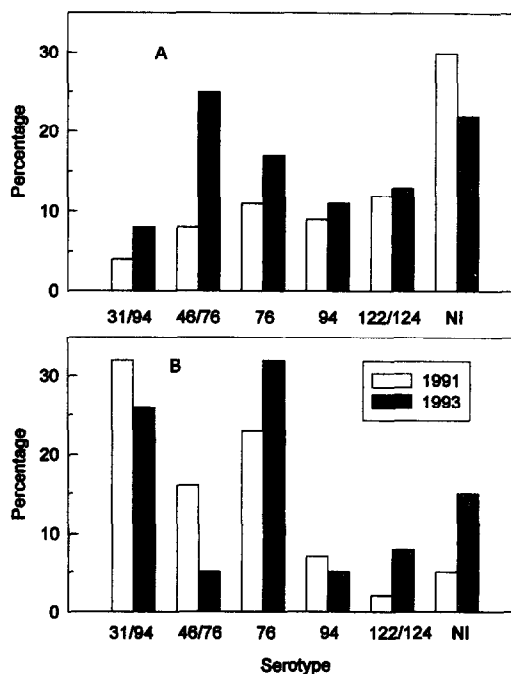


Fig. 1. Distribution of selected serotypes in nodules from soybean plants grown in a Cape Fear (A) and a Dothan (B) soil in 1991 and 1993. The distribution is based on analysis of 480 nodules from each site in 1991 and 64 nodules from each site in 1993. NI = not identified serologically.
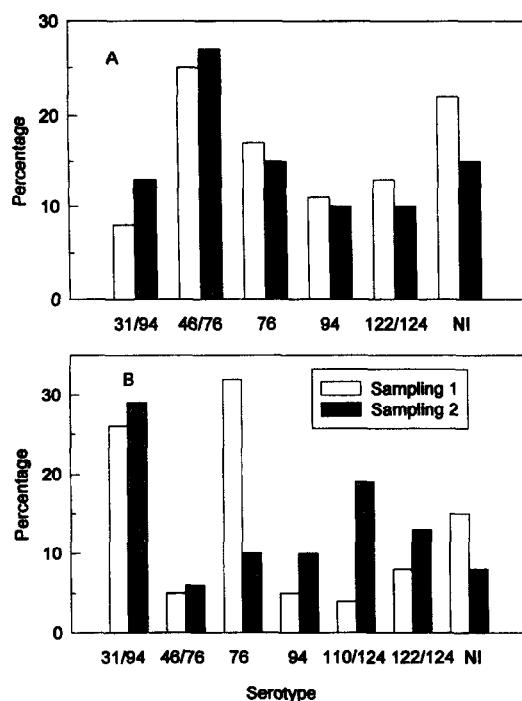
Fig. 2. The effect of plant growth stage on the distribution of selected serotypes in nodules from soybean plants grown in a Cape Fear (A) and a Dothan (B) soil. Distributions are based on analysis of 64 nodules from each site at each sampling. Sampling 1 occurred at the late vegetative growth stage and sampling 2 occurred at the mid podfill growth stage. NI = not identified serologically.

minor serotypes pooled as a group for the two different years revealed that serotype distributions changed significantly between growing seasons ($P = 0.05$). The greatest change was the increase in occurrence of serotype 46/76. In the Dothan soil, serotypes 31/94, 46/76, 76 and 94, which were predominant in nodules in 1991, also occurred in a high frequency in 1993. Although differences in the distribution of serotypes were observed (Fig. 1B), they were not significant ($P = 0.05$) by the $\chi^2$ analysis.

The occurrence of serotypes in nodules of plants at late vegetative and early podfill growth stages (1993) was the same for plants grown in the Cape Fear soil (Fig. 2A). However, for plants grown in the Dothan soil (Fig. 2B), there was a significant change ($P = 0.05$) in the distribution of serotypes,

most notably a reduction in the occurrence of serotype 76 and an increase in occurrence of serotype 110/124.

### Resistance to antibiotics

The antibiotic resistances of isolates were classified into three minimal inhibitory concentration (MIC) categories (Table 3). Isolates from both soils were found in all three MIC categories. A higher percentage (78%) of the Dothan soil isolates resisted high levels of antibiotics than isolates from the Cape Fear soil (47%). In most cases isolates with the same serotype were classified in the same MIC category (Table 4). However, diversity in this characteristic was observed for isolates with serotypes 76 and 122/124 from the Cape Fear soil. The non-identified isolates from the Cape Fear soil exhibited greater diversity in antibiotic resistance patterns (assigned to all three MIC categories) than the non-identified isolates from the Dothan soil.

### $N_2$-fixing capacity

The $N_2$-fixing capacity of the native isolates varied considerably. However, two-thirds of isolates from both locations (66–67%) had intermediate $N_2$-fixation capacity (Fig. 3). Less than 15% of the isolates (12–14%) at both locations were classified as having high $N_2$-fixation capacity, while about 20% had low $N_2$-fixation capacity, i.e. fixed similar amounts of $N_2$ as the reference strain USDA 31.

### Yield response to nitrogen

Seed yield of the non-nodulating cultivar increased significantly on both soils with the application of 150 kg N ha$^{-1}$ (Table 5). This indicated a limiting supply of residual N in these soils. Application of 150 kg N ha$^{-1}$ also significantly increased the seed yield of the unimproved cultivar, Arksoy, in the Dothan soil but not in the Cape Fear soil (Table 5). Seed yields of the four improved nodulating cultivars, which are descendants of Arksoy, were not increased significantly by application of 150 kg N ha$^{-1}$ (Table 5).

### DISCUSSION

Although differences among plant genotypes in their acceptance of *Bradyrhizobium* strains have

Table 3. Antibiotic resistance of reference strains and soybean bradyrhizobia from a Cape Fear and a Dothan soil in North Carolina

| Category | MIC ($\mu$gml$^{-1}$)* | | | Reference strains | Origin of isolate† | |
|---|---|---|---|---|---|---|
| | Ery | Spec | Strep | | Cape Fear | Dothan |
| I | 100–200 | 200–400 | 200–400 | USDA 31,46,76,94 | 47 | 78 |
| II | 200 | 100 | 50–200 | USDA 122 | 6 | 11 |
| III | 13–100 | 13–50 | 13–50 | USDA 24 MN110 NC 1028 | 47 | 11 |

*MIC = Minimal inhibitory concentration of erythromycin, spectinomycin and streptomycin.
†Percentage of isolates in each category per location.

Table 4. Characteristics of soybean bradyrhizobia isolated from nodules of plants grown in a Cape Fear and a Dothan soil

| Serotype | MIC category* | $N_2$-Fixing capacity† | Number | |
|---|---|---|---|---|
| | | | Cape Fear | Dothan |
| 24 | III | Intermediate | 1 | 2 |
| | | Low | 1 | 0 |
| 31/94 | I | High | 0 | 1 |
| | | Intermediate | 1 | 8 |
| | | Low | 2(2Ch‡) | 6(5Ch‡) |
| 46/76 | I | High | 3 | 3 |
| | | Intermediate | 6 | 4 |
| 76 | I | Intermediate | 5 | 9(2Ch) |
| | | Low | 0 | 1(1Ch) |
| | III | Intermediate | 1 | 0 |
| 94 | III | Intermediate | 5 | 2 |
| | | Low | 0 | 1 |
| 122/124 | II | High | 0 | 2 |
| | | Intermediate | 1 | 0 |
| | III | High | 2 | 0 |
| | | Intermediate | 3 | 0 |
| | | Low | 1 | 0 |
| 1001/1028 | III | Intermediate | 1 | 0 |
| | | Low | 1 | 0 |
| NI§ | I | Intermediate | 3 | 1 |
| | | Low | 0 | 1 |
| | II | Intermediate | 1 | 3 |
| | | Low | 1 | 0 |
| | III | Intermediate | 1 | 0 |
| | | Low | 3 | 0 |

*MIC = minimal inhibitory concentration.

†High: $N_2$ fixed $> 100$ mg N plant$^{-1}$; Intermediate: $N_2$ fixed $< 100$ but $> 64$ mg N plant$^{-1}$; Low: $N_2$ fixed $< 64$ mg N plant$^{-1}$. The efficient control, strain MN110, fixed 119 mg N plant$^{-1}$ and the inefficient control, strain USDA 31, fixed 45 mg N plant$^{-1}$. The $LSD_{0.05}$ is 19 mg N plant$^{-1}$.

‡Number of isolates that induced leaf chlorosis.

§NI = not identified by serological reaction.

been reported (Cregan *et al.*, 1989 and Weiser *et al.*, 1990), differences were not observed in this study. The numbers of serotypes observed with each cultivar (11–14 for the Dothan soil; 16–21 for
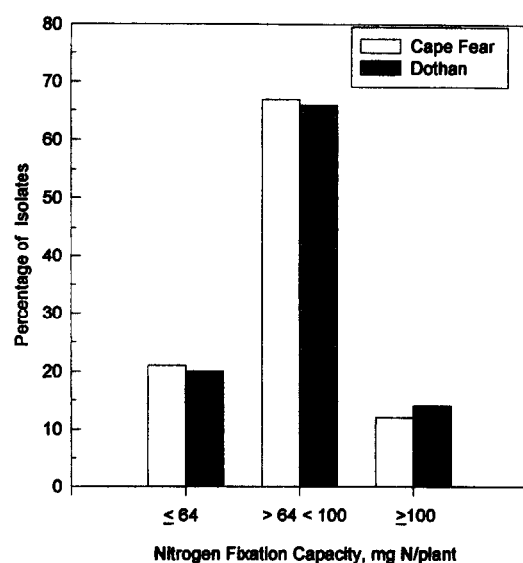


Fig. 3. Symbiotic $N_2$-fixing efficiency of soybean bradyrhizobial isolates from a Cape Fear soil and a Dothan soil. Forty-three isolates from the Cape Fear soil and 44 isolates from the Dothan soil were tested. $N_2$ fixed equals 119 mg N plant$^{-1}$ for the efficient control strain, MN110, and 45 mg N plant$^{-1}$ for the inefficient control strain, USDA 31. The $LSD_{0.05}$ is 19 mg N plant$^{-1}$.

the Cape Fear soil) are in the same range observed by Mpepereki and Wollum (1991) (10–17 serotypes) in five soils of North Carolina. The total number of serotypes identified increased substantially (24 for the Dothan soil, 46 for the Cape Fear soil), when the data from the five soybean cultivars were combined. This might be related either to the use of different soybean cultivars as trap hosts, or the larger sample size. The total number of serotypes might be somewhat overestimated since some nodules may have been occupied by two different strains. However, Mpepereki and Wollum (1991) have shown that single cell isolates can have the same multiple reactions as the nodules from which they were isolated. This suggests occupancy by unique serotypes with multiple antigens. The greater diversity in the Cape Fear soil population than in the Dothan soil population is evident not only by the larger number of identified serotypes but also by the smaller number of nodules classified in each category. One of the major differences between the soils is the 10-fold higher organic matter content of the Cape Fear soil (Table 1). A greater number and higher concentrations of substrates and a lower desiccation potential may have contributed to the greater diversity of the bradyrhizobial population in the Cape Fear soil.

The general trend observed over the two years of sampling was for serotypes 46/76, 76, 94, 122/124 and occasionally 31/94 to be most frequent in the

Table 5. Yield response of soybean cultivars to application of inorganic N when grown in two Coastal Plain soils

| Soil type | Cultivar | Seed yield (Mg ha$^{-1}$)* | |
|---|---|---|---|
| | | Minus N | 150 kg N ha$^{-1}$ |
| Cape Fear | Arksoy | 2.03 | 1.81 |
| | Davis | 2.92 | 2.76 |
| | Young | 3.09 | 3.13 |
| | Brim | 3.30 | 3.33 |
| | N90-852 | 3.51 | 3.26 |
| | Nonnod | 0.88 | 2.00 |
| | $LSD_{05}^{\dagger}$ | 0.35 | |
| Dothan | Arksoy | 2.83 | 3.43 |
| | Davis | 3.57 | 3.76 |
| | Young | 3.68 | 3.60 |
| | Brim | 3.93 | 3.81 |
| | N90-810 | 3.78 | 3.90 |
| | Nonnod | 1.40 | 2.59 |
| | $LSD_{05}$† | 0.38 | |

*Yields were corrected to 13% moisture.
†Since $LSD_{05}$ values are based on a significant cultivar by N interaction, they can be used to compare any two treatment means for the same soil.

Cape Fear soil and for serotypes 31/94, 46/76, 76, 94 and occasionally 122/124 to be predominant in the Dothan soil. Most of these serotypes have been observed in other soils of North Carolina (Weber et al., 1989; Mpepereki and Wollum, 1991). Factors such as previous inoculations, soil physical–chemical properties, competition with other microorganisms present, and temperature might interact to produce a specific serotype distribution in soybean nodules at each location (Dowling and Broughton, 1986; Barnet, 1991).

While most serotypes occupied similar percentages of the total nodule population sampled within and across seasons, considerable variation in occupancy was noted for specific serotypes. For example, nodule occupancy by serotype 46/76 in the Cape Fear soil increased from 8% in 1991 to 25% in 1993 (Fig. 1A) and nodule occupancy by serotype 110/124 in the Dothan soil increased from 4% at the vegetative growth stage to 19% at the mid podfill stage (Fig. 2B). The latter result is of particular interest since serotype 110/124 occurred in less than 2% of the nodules sampled in 1991. Similar seasonal variations of Bradyrhizobium japonicum serotypes in nodules of soybean were observed by Moawad et al. (1984). Such instability in the distribution of serotypes in the nodules should be taken into account when selecting inoculant strains. Successful inoculant strains need to compete against different native strains that infect the plant at different stages of growth.

The antibiotic resistance patterns were useful for delineating isolates not identified by serological reactions. They also revealed a greater diversity among 76 and 122/124 isolates from the Cape Fear soil than among isolates with similar serotypes from the Dothan soil. While 78% of the bradyrhizobial

isolates from the Dothan soil resisted high concentrations of antibiotics, only 47% in the Cape Fear soil were resistant (Table 3). Selective pressures in the Dothan soil apparently resulted in the evolution of a population composed of strains with greater antibiotic resistance, which may be Bradyrhizobium elkani (Kuykendall et al., 1992).

The majority of nodules from both soils were classified as serotypes 31/94, 46/76, 76 or 94, which are the serotypes observed for the reference strains USDA 31, 46, 76 and 94, respectively, in this study. Rhizobitoxine symptoms in soybean have been associated with bradyrhizobia that have antigenic reactions similar to those of these USDA reference strains (Devine et al., 1988; Fuhrmann, 1990; Minamisawa and Fukai, 1991). Production of rhizobitoxine as evidenced by observation of leaf chlorosis was observed in the greenhouse experiment for isolates with serotype 31/94 and 76 (Table 4). In another experiment (results not shown), the symptoms were also observed in strains with serotype 94. However, isolates with serotype 46/76 from the Cape Fear soil or the Dothan soil never induced chlorotic leaves. Furthermore, some of these isolates have high $N_2$-fixation capacity (Table 4). These results corroborate observations of others (Basit et al., 1991; Judd et al., 1993) that similarity in antigenic properties does not necessarily imply similarity in other phenotypic or genotypic properties. In field experiments, Vasilas and Fuhrmann (1993) and Mahler and Wollum (1981) showed that inoculation with rhizobitoxine-producing strains caused poor plant development and reduction in seed yield. In the Cape Fear and Dothan soils the presence of chlorosis-inducing strains in the bradyrhizobial populations did not have any apparently detrimental effect on plant growth and yield. Preliminary experiments (results not shown) revealed that of the cultivars tested, only Brim and N90-852 were sensitive to native rhizobitoxine-producing strains.

The majority of isolates from both soils were classified as having intermediate $N_2$-fixation capacity (Fig. 3) and up to 50% of nodule occupants in the Dothan soil had serotypes (31/94 and 76) associated with rhizobitoxine production (Table 4). Yield trials on the same sites and in the same year that nodules were sampled for serological analysis and isolation of occupants indicated that native populations supported seed yields of four improved nodulating cultivars that ranged from 3.2 to 3.7 Mgs ha$^{-1}$. The yield of these cultivars was not increased significantly by application of 150 kg N ha$^{-1}$ (Table 5). The large response of the non-nodulating cultivar to N application (Table 5) indicates that residual soil N was yield limiting to plants that could not fix $N_2$. In addition, $N_2$ fixation was shown to contribute 62–72% of the crop N requirement in the absence of N fertilization (Israel and Burton, 1997). These results indicate

that, even though the majority of the bradyrhizobial isolates from these soils had undesirable attributes, crop yield under the existing environmental conditions was not limited by the overall symbiotic $N_2$-fixation capacity of the indigenous bradyrhizobial populations.

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